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1. Your reference — SMC 60378/GB/P

2. Patent application number *(The Patent Office will fill in this part)* — 03 SEP 1999 — **9920774.8**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

AVECIA Limited
Hexagon House
PO Box 42
Blackley
Manchester, M9 8ZS

Patents ADP number *(if you know it)*

6254007002

7698 48300

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

Polymer

5. Name of your agent *(if you have one)*

FAWKES, David Melville

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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773278.7 GB

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Number of earlier application	Date of filing *(day / month / year)*

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Signature Kathy Pinder Date 3/9/99

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SMC 60378

POLYMER

The present invention relates to polymeric antimicrobial compounds which carry a chromophoric marker, more particularly to cationic polymeric antimicrobial compounds carrying a covalently bound chromophoric marker. The invention also relates to methods for detecting the polymeric antimicrobial compounds.

Polymeric antimicrobial compounds are used in a wide range of applications requiring the control or elimination of micro-organisms, for example as preservatives, disinfectants, slimicides, algicides and in water treatments such as cooling water and swimming pools. Polymeric antimicrobial compounds, especially cationic polymeric antimicrobial compounds are particularly useful and offer a number of advantages over molecular quaternary ammonium compounds, because they are of relatively low toxicity and exhibit reduced foaming when added to a liquid medium such as water.

To prevent the proliferation of micro-organisms in a medium containing an antimicrobial compound it is necessary to ensure that the concentration of antimicrobial compound is sufficient to give an antimicrobial effect in the medium. However, in some media, especially in swimming pools, the concentration of antimicrobial compound reduces with time. This can result in loss of the antimicrobial effect and the subsequent proliferation of micro-organisms. The concentration of an antimicrobial compound in a medium can reduce via a number of different mechanisms, for example, through interaction of the antimicrobial compound with micro-organisms or with other components present in the medium.

To ensure that a medium remains protected by the antimicrobial compound it is therefore important that the concentration of antimicrobial compound in, or on, a medium can be accurately determined. Generally antimicrobial compounds are used at very low concentrations in a medium, often less than 10 ppm. It is therefore important that the concentration of the antimicrobial compound can be accurately determined to ensure that sufficient antimicrobial compound is present in the medium.

In some applications there is a need to detect the presence of the antimicrobial compound at even lower levels. For example antimicrobial compounds are often used to treat fruit. However, before the fruit is consumed it is necessary to wash the fruit to remove the antimicrobial compounds from the fruit. Typically the washing process is required to reduce the concentration of the antimicrobial compounds to about 1 to 10 ppb. In this application it is therefore necessary to measure the concentration of antimicrobial material down to ppb levels.

The polymeric nature of polymeric antimicrobial compounds makes accurate determination of the concentration of these compounds difficult and time consuming. This

is especially true of cationic polymeric antimicrobial compounds because the cationic groups tend to associate themselves with a surface to which they are applied. To determine the concentration of the antimicrobial compound on a surface, for example on the surface of fruit, it is necessary to extract the antimicrobial compound from the surface and analyse the extract, for example using gel permeation chromatography. However, because most antimicrobial polymers comprise a mixture of polymer chains of different lengths, this procedure often gives a misleading result of the concentration because the extraction method tends to preferentially extract the shorter polymer chains.

Furthermore, polymeric antimicrobial compounds are often used in media which contain numerous other components which can interfere with the analysis method used to estimate the concentration of the polymeric antimicrobial compound. For example, in swimming pools poly(hexamethylenebiguanide) (PHMB) is commonly used as a primary sanitizer. A known colorimetric method for estimating the PHMB in the pool is based on the interaction of PHMB with bromophenol blue or Eosin dyestuffs. However, this test also detects molecular quaternary ammonium compounds which are often present in swimming pools and thereby gives a false measure of concentration of the PHMB.

We have found that by covalently binding a chromophoric marker on, or in, an antimicrobial polymer enables the antimicrobial compounds to be detected with greater accuracy, especially at low concentration without adversely affecting the antimicrobial properties of the polymer.

According to a first aspect of the present invention there is provided an antimicrobial polymer, characterised in that it carries a covalently bound chromophoric marker (hereinafter "The Polymer").

Preferably The Polymer is a cationic antimicrobial polymer and especially a polyquaternary ammonium compound or a polymeric bisbiguanide.

The chromophoric marker comprises a chromophoric group which absorbs and/or emits radiation at wavelengths characteristic of the chromophoric group. The wavelength of absorbtion and/or emission provides a reproducible "signature" associated with The Polymer by means of which it is possible to detect the presence of The Polymer using a suitable optical or spectroscopic detection method. This signature is preferably different from any absorption bands inherent in the antimicrobial polymer which does not contain the chromophoric marker.

Preferably the chromophoric group has a major absorption and/or emission band in the range of from 390 to 1100 nm, more preferably from 400 to 800 nm.

The chromophoric group preferably comprises an azo, anthraquinone, pyrroline, phthalocyanine, polymethine, aryl-carbonium, triphenodioxazin , diarylmethane, triarylmethane, anthraquinone, phthalocyanine, methine, polymethine, rhodamine,

indoaniline, indophenol, stilbene, squarilium, coumarin, aminoketone, xanthene, fluorine, acridene, acridan, acridinium, quinolene, thiazole, azine, nigrosine, oxazine, thiazine, indigoid quininoid, quinacridone, lactone, pyrroline, luciforyl, indacene, benzodifuranone, or indolene group or a combination of such groups.

In a preferred embodiment of the present invention the chromophoric group is a fluorescent group which emits radiation in a specific fluorescence band at a wavelength which is longer than that of the absorption band. Preferably the fluorescent group has its major absorption band of in the range of from 390 to 1100 nm, more preferably from 400 to 800 nm. Preferably the fluorescence band is from 420 to 480 nm, more preferably from 440 to 460 nm.

Preferred fluorescent groups include phthalocyanine; methine; croconium; stilbene, for example 4-acetamido-4'-isothiocyanatostilbene; coumarin, for example 7-amino-4-methylcoumarin and 7-amino-4-trifluoromethylcoumarin; acridan; acridinium; luciforyl; squarylium; indacene, for example 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; and xanthene, for example Rhodamine B, Rhodamine 6G, Rhodamine 123, Fluorocein, 5-amino-Fluorocein and 5-(4,6-dichlorotriazin-2-yl)amino-Fluorocein. It is especially preferred that the fluorescent group is a 1,8-naphthalimide derivative.

In another preferred embodiment, the chromophoric group is one which produces a characteristic Raman spectrum when irradiated with monochromatic light. Preferred chromophores in this embodiment are rhodamines and azo dyes, especially Rhodamine 6G.

The chromophoric marker may be covalently bound to the antimicrobial polymer as a pendant group or a terminal group on the polymer chain or, most preferably as an in-chain group in the polymer chain.

When the chromophoric marker is present as a pendant or terminal group on the polymer chain, the covalent bond between the polymer and the marker is preferably formed by means of one or more reactive functional groups on the chromophoric marker which is capable of forming a covalent bond with the polymer and/or monomer precursors used to make the polymer.

When the chromophoric marker is incorporated into the polymer chain the chromophoric marker preferably has two or more reactive functional groups which are capable of forming a covalent bond with one or more of the monomers, or chain segments, used to prepare the polymer and is thereby incorporated covalently as a component in the polymer chain. For example the chromophoric marker may be incorporated into The Polymer by means of an ester, ether, amide or urethane group.

The reactive functional group(s) carried by the chromophoric marker may be any functionality which is capable of reacting with the antimicrobial polymer or a monomer

used in the preparation of the antimicrobial polymer. Preferred reactive functional groups include -OH, -NHR^1, -NH-, -SH, -$COOR^1$, epoxy, alkenyl, isocyanate, thioisocyanate or a halogen atom, wherein R^1 is H or optionally substituted alkyl. More preferably the reactive functional group is -NH_2, -OH, -SH, -NCO or -NCS. It is especially preferred that the reactive functional group is -NH_2, -NCO or -NCS, more especially -NH_2.

The reactive functional group(s) may be attached directly to the chromophoric group or, more preferably, through a linker group.

Preferred linker groups are aliphatic (preferably alkylene or alkenylene), arylene, heteroarylene or a combination thereof. When the linker group is aliphatic it preferably contains up to 10 carbon atoms. The aliphatic group may be branched but is preferably a straight chain group. Preferably the aliphatic group is a C_{1-10}-alkylene or a C_{2-10}-alkenylene group and especially a C_{2-6}-alkylene group. The aliphatic group may also contain one or more hetero atoms selected from O, S and N.

When the linker group is an aryl group it is preferably naphthylene or more preferably phenylene.

When the linker group is heteroarylene it is preferably a triazinylene or pyrimidinylene group.

The linker group may comprise a combination of linker groups. For example the linker may comprise an alkyleneamino group attached to a triazinyl or pyrimidinyl group attached to the chromophoric group by a reactive functional group.

An especially preferred chromophoric marker which carries reactive functional group(s) is of the Formula (1):

$$Ch\text{-}[(L)_nX]_p$$

Formula (1)

wherein:

Ch is a chromophoric group;
L is an aliphatic linking group;
X is a reactive functional group;
n is 0 or 1; and
p is 1 or 2.

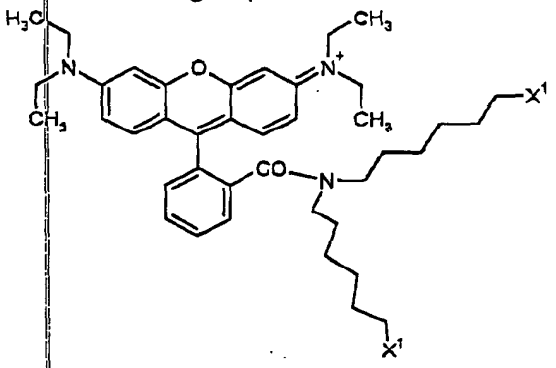
Preferably L is C_{2-6}-alkylene. n is preferably 1. X is preferably -OH, -NH_2 or -SH.

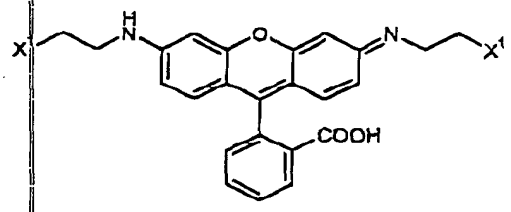
When the chromophoric marker is attached to the polymer as a terminal or pendant group, p is 1. When the chromophoric marker is attached as an in-chain group in the polymer, p is 2.

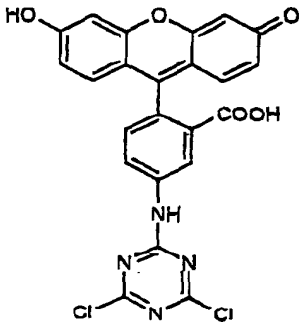
Preferred chromophoric groups represented by Ch are the hereinbefore defined preferred chromophoric groups.

Preferred examples of chromophoric markers which carry reactive functional group(s) attached directly to the chromophore include 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulphonic acid, 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid and tetramethylrhodamine isothiocyanate.

Examples of chromophoric markers carrying reactive functional groups attached to a chromophoric group via linking group(s) include N-(6-aminohexyl)-4-(6-aminohexylamino)-1,8-naphthalimide and groups of the formulae:





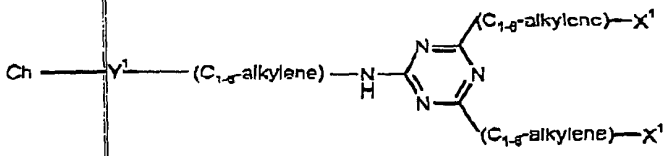


wherein:

Ch is a chromophoric group; and

X^1 is a reactive functional group as hereinbefore defined.

A preferred chromophoric group carrying reactive functional groups attached by means of an alkyleneamino triazinyl group to the chromophoric group is of the formula:

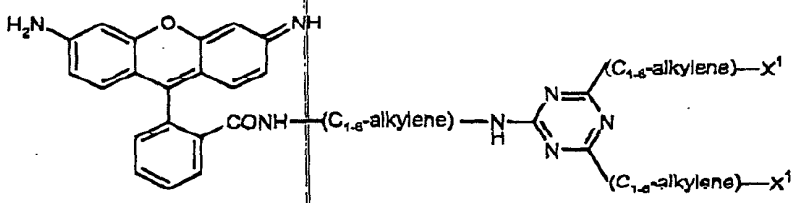


wherein:

Y^1 is NH, CONH or S; and

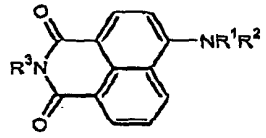
Ch and X^1 are as hereinbefore defined.

An especially preferred group of the above formula is of the formula:



wherein X^1 is as hereinbefore defined.

In an especially preferred embodiment the chromophoric marker is of the Formula (2):



Formula (2)

wherein:

R^1 is H or C_{1-6}-alkyl optionally substituted by amino, hydroxy or mercapto; and

R^2 and R^3 are, independently, alkyl substituted by a reactive functional group.

R^1 is preferably H.

R^2 and R^3 are preferably C_{1-10}-alkyl, more preferably C_{1-8}-alkyl, especially C_{2-6}-alkyl and more especially hexyl substituted by a reactive functional group. Preferred reactive functional groups on R^2 and R^3 are the reactive functional groups represented by X^1 as hereinbefore defined, more preferably $-NH_2$, -SH, -NCO or -NCS and especially $-NH_2$. For ease of synthesis it is preferred that R^2 and R^3 are the same.

An especially preferred compound of the Formula (2) is N-(6-aminohexyl)-4-(6-aminohexylamino)-1,8-naphthalimide (alternatively 2-(6-aminohexyl)-6-(6-aminohexylamino)-benzo[de]isoquinoline-1,3-dione).

The compounds of Formula (2) are especially useful for incorporating the chromophoric group into the polymer chain of The Polymer.

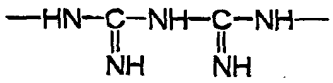
The compounds of Formula (2) are believed to be novel and form a further aspect of the present invention. The compounds of Formula (2) may be prepared by reacting 4-bromo-1,8-naphthalic anhydride with 1 molar equivalent of a compound of the formula NHR^3 followed by reaction with 1 molar equivalent of a compound of the formula NHR^1R^2, wherein R^1, R^2 and R^3 are as hereinbefore defined.

Preferably the chromophoric marker is present in The Polymer at a concentration which is insufficient to significantly affect the anti-microbial properties of the polymeric material compared to a polymer without the chromophoric marker. Preferably the chromophoric marker is present at less than 10%, more preferably less than 5% and especially less than 1% based upon the total weight of The Polymer.

The antimicrobial polymer to which the chromophoric marker bound may be any antimicrobial polymer, preferably a cationic antimicrobial polymer, more preferably an antimicrobial poly(quaternary ammonium) compound or a polymeric guanide and especially a polymeric biguanide.

Preferred antimicrobial poly(quaternary ammonium) compounds to which the chromophoric group is covalently bound include, for example poly[oxyethylene(dimethyliminio)ethylene(dimethyliminio)ethylene dichloride], poly[hydroxyethylene(dimethyliminio)ethylene(dimethyliminio)methylene dichloride] and a copolymer obtainable by copolymerising 1,2-ethylenediamine, (chloromethyl)oxirane and N-methyl amine (commercially available as Busan 1157 from Buckman Laboratories in the USA).

When the chromophoric marker is attached as a pendant or terminal group on a polymeric biguanide, the polymeric biguanide to which it covalently bound contains at least one biguanide unit of Formula (3):

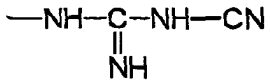


Formula (3)

Preferably, the polymeric biguanide contains at least two biguanide units of Formula (3) which are linked by a bridging group which contains at least one methylene group. The bridging group may include a polymethylene chain which may optionally be interrupted by hetero atoms such as oxygen, sulphur or nitrogen. The bridging group may

include one or more cyclic nuclei which may be saturated or unsaturated. Preferably, the bridging group is such that there are at least three, and especially at least four, carbon atoms directly interposed between two adjacent biguanide units of Formula (3). Preferably, there are not greater than ten and especially not greater than eight carbon atoms interposed between two adjacent biguanide units of Formula (3).

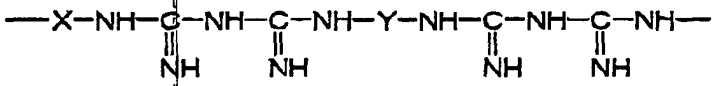
The polymeric biguanide may be terminated by any suitable group which may be a hydrocarbyl or substituted hydrocarbyl group or an amine or a group



When the terminating group is a hydrocarbyl group, it may be alkyl, cycloalkyl or aralkyl.

When the terminating group is a substituted hydrocarbyl group, the substituent may be any substituent that does not exhibit an undesirable adverse effect on the microbiological properties of the polymeric biguanide. Examples of such substituents or substituted hydrocarbyl groups are aryloxy, alkoxy, acyl, acyloxy, halogen and nitrile.

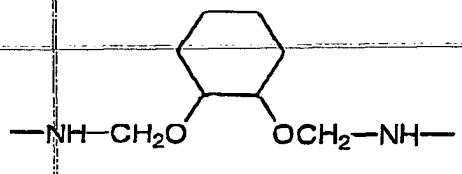
The polymeric biguanide preferably contains more than two biguanide units of Formula (3) and preferably is a linear polymeric biguanide which has a recurring polymeric unit represented by Formula (4)



Formula (4)

wherein X and Y may be the same or different and represent bridging groups in which, together, the total number of carbon atoms directly interposed between the pairs of nitrogen atoms linked by X and Y is not less than 9 and not greater than 17.

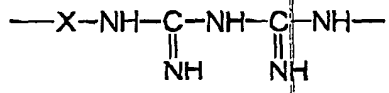
The bridging groups X and Y may consist of a polymethylene chain, optionally interrupted by a heteroatom such as oxygen, sulphur or nitrogen. X and Y may also incorporate a cyclic nucleus which may be saturated or unsaturated, wherein the number of carbon atoms directly interposed between the pairs of nitrogen atoms linked by X and Y is taken as including that segment of the cyclic group, or groups, which is the shortest. Thus, the number of carbon atoms directly interposed between the nitrogen atoms in the group



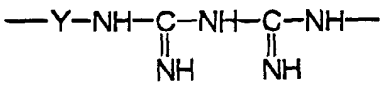
is 4 and not 8.

The preferred polymeric biguanide for use in the present invention is poly(hexamethylenebiguanide), in which both X and Y in Formula 4 are the group $-(CH_2)_6-$

The polymeric biguanides of Formula 4 are typically obtained as mixtures of polymers in which the polymer chains are of different lengths. Preferably, the number of individual biguanide units

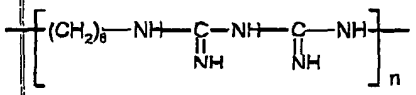


and



is, together, from 3 to about 80.

In the case of the preferred poly(hexamethylenebiguanide) it is a mixture of poly(hexamethylenebiguanide) polymer chains in which the individual polymer chains, excluding the terminal groups, are represented by Formula (5) and salts thereof:



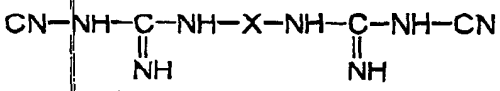
Formula (5)

wherein the value of n is from 4 to 40 and especially from 4 to 15. It is particularly preferred that the average value of n in the mixture is 12. Preferably, the average molecular weight of the polymer mixture is from 1100 to 3300.

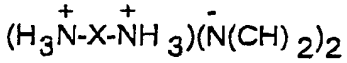
When the chromophoric marker is present as an in-chain group in The Polymer, The Polymer is obtainable by co-polymerising the chromophoric marker with the monomers used to prepare the antimicrobial polymer which does not contain the chromophoric marker. For example, a polymeric quaternary ammonium antimicrobial material obtainable by co-polymerising 1,2-ethlenediamine, (chloromethyl)oxirane, N-methyl amine and a chromophoric marker as hereinbefore defined.

In a preferred embodiment of the present invention The Polymer is a polymeric biguanide wherein the chromophoric marker is incorporated into the polymer chain.

In this preferred embodiment The Polymer is obtainable by the copolymerisation of a chromophoric marker, a bisdicyandiamide having the formula:



and a diamine $H_2N\text{-}Y\text{-}NH_2$, wherein X and Y have the meanings defined above. Alternatively The Polymer is obtainable by coplomerisation of a chromophoric marker, a diamine salt or dicyanimide having the formula:



and a diamine H_2N-Y-NH_2 wherein X and Y have the meanings defined above. These methods of preparation are analogous to those described in UK specifications numbers 702,268 and 1,152,243 respectively. Any of the polymeric biguanides described in GB 702,268 and GB 1,152,243 may be prepared with a chromophoric marker present in the polymer chain by addition of a chromophoric marker during the copolymerisation of the monomers used to prepare the polymeric biguanides described therein.

It is especially preferred that The Polymer is obtainable by co-polymerising hexamethylenediamine, hexamethylene-1,6-bis dicyandiamide (HMBDA) and a chromophoric marker.

In this preferred embodiment the chromophoric marker is preferably of the Formula (2) as hereinbefore defined.

In yet another preferred embodiment The Polymer is obtainable by co-polymerising hexamethylenediamine, hexamethylene-1,6-bis dicyandiamide (HMBDA) and 1,8-naphthoic anhydride.

It is believed that during the copolymerisation the 1,8-naphthoic anhydride reacts with 2 molar equivalents of the hexamethylene diamine to give N-(6-aminohexyl)-4-(6-aminohexylamino)-1,8-naphthalimide. The 1,8-naphthalimide groups are thereby incorporated into The Polymer as an in-chain chromophoric marker.

In these preferred embodiments the co-polymerisation of the hexamethylenediamine, hexamethylene-1,6-bis dicyandiamide (HMBDA) and a chromophoric marker containing reactive functional groups is preferably performed at a temperature of from 80 to 200°C, more preferably 110 to 170°C and especially from 120 to 160°C. The molar ratio of HMBDA to hexamethylenediamine is preferably approximately 1:1

When The Polymer is cationic, it may be used in free base form but is preferably used in the form of a salt with an acid. Preferred salts are those with an inorganic acid, especially the hydrochloride salt, and salts with organic acids. Preferred salts with organic acids are those with organic carboxylic acids, preferably carboxylic acid with from 4 to 20 carbon atoms (excluding the carbon of the carboxyl group), for example the stearate salt.

In an embodiment of the present invention The Polymer is present in admixture with one or more antimicrobial polymers which do not contain a chromophoric marker. The Polymer may be, apart from the marker, different from the antimicrobial polymer which does not contain the chromophobic marker, but is preferably the same. Such mixture may arise during manufacture wherein the amount of the chromophoric maker relative to the antimicrobial polymer, or precursor chain segments or monomers, is less

than that required to give a mixture of antimicrobial polymers wherein each polymer contains one or more chromophoric markers. Alternatively, the mixture of polymers may arise from mixing together The Polymer and an antimicrobial polymer which does not contain the antimicrobial marker. In this instance The Polymer constitutes a master batch concentrate. The amount of the chromophoric marker to antimicrobial polymer may, therefore, vary over wide limits. At one extreme, the mixture of antimicrobial polymers contains sufficient polymers containing the chromophoric marker to allow for detection of the mixture at the ppb level and at the other extreme the mixture of polymers contains only polymers which contain the chromophoric marker group.

According to a further aspect of the invention there is provided a composition comprising antimicrobial polymers at least some of which contain a chromophoric marker.

Preferably, in this embodiment, the amount of the chromophoric marker is not greater than 10%, more preferably not greater than 1%, even more preferably not greater than 0.01% and especially not greater than 0.001%, by weight, based on the amount of antimicrobial polymers.

According to a second aspect of the present invention there is provided a composition comprising a carrier and The Polymer.

The carrier may be a solid but is preferably a liquid.

The liquid may be water, a polar organic solvent or a mixture thereof.

When the carrier is water, the aqueous composition may also contain other adjuvants which help distribute The Polymer uniformly throughout the composition. Examples of such adjuvants are compounds which provide structure to the water to inhibit sedimentation such as alginates and gums, particularly Xanthan gum.

By the term "polar" in relation to the organic solvent is meant an organic liquid or resin capable of forming moderate to strong bonds as described in the article entitled "A Three Dimensional Approach to Solubility" by Crowley et al in Journal of Paint Technology, Vol. 38, 1966, at page 269. Such organic liquids generally have a hydrogen bonding number of 5 or more as defined in the above mentioned article.

Examples of suitable polar organic liquids are amines, ethers, especially lower alkyl ethers, organic acids, esters, ketones, glycols, alcohols and amides. Numerous specific examples of such moderately strongly hydrogen bonding liquids are given in the book entitled "Compatibility and Solubility" by Ibert Mellan (published in 1968 by Noyes Development Corporation) in Table 2.14 on pages 39-40 and these liquids all fall within the scope of the term polar organic liquid as used herein.

The Polymer and compositions according to the present invention may be used to protect various media from microbiological growth.

According to a third aspect of the invention there is provided a method for inhibiting microbiological growth on, or in a medium which comprises treating the medium with The Polymer. The Polymer can be used in any conditions in which micro-organisms grow and cause problems. Thus, the medium may be an industrial medium such as a cooling water tower liquid, paper mill liquor, metal working fluid, geological drilling lubricant, polymer emulsion, surface coating composition such as paint, varnish or lacquer. The medium to be protected can be a solid such as wood or leather and particularly solid surfaces in the health-care or food preparation industries. The solid may also be a textile material such as cellulose, including its blends with synthetic polymers and also non-woven materials such as those used in disposable items such as nappies, incontinence pads and feminine hygiene packs.

The Polymer and compositions thereof according to the invention may also be used in personal care formulations which are of many types and include water-in-oil and oil-in-water emulsions. Many of these personal care formulations involve applications to the skin and include, inter alia, hand lotions, foundation creams, emollient creams, facial washing creams, shaving creams, after-shave lotions, sunscreen lotions and creams, sunscreen hair protectors, after-sun lotions, antiperspirants, deodorants, hair gels, hair colorants, hair mousse, mascara, eye shadows, eye liners, lipstick, lip gloss, facial blusher, rouge, foundations and fragrances, shampoo, shampoo gel, conditioning rinse, toothpaste, mouthwash, foam bath liquid, soluble bath oil and liquid soap formulations.

Where the medium to be protected is a solid, The Polymer may be applied by any method known to the art such as spraying, dipping or coating with a composition containing The Polymer.

As noted hereinbefore, the amount of The Polymer which is applied to the medium to be protected from microbiological growth may be just sufficient to inhibit such growth or it may be in excess of such amount. Preferably, the amount of The Polymer which is applied to such medium is not greater than 2% and more preferably not greater than 1% by weight of the medium. Generally, adequate protection is provided by from 1 ppm to 500 ppm, particularly 10 to 200 ppm and especially 10 to 100 ppm of The Polymer relating to the medium.

The Polymer according to the present invention may be used alone or in combination with one or more further antimicrobial compound so as to increase the antimicrobial spectrum of activity. When The Polymer is used with another antimicrobial compound the components of such a mixture preferably provide a synergistic increase in antimicrobial effectiveness compared with the individual antimicrobial compounds in the composition. Indeed, many of the following examples exhibit synergism with The Polymer.

Examples of antimicrobial compounds which may be used with The Polymer include one or more of quaternary ammonium compounds such as N.N-diethyl-N-dodecyl-N-benzylammonium chloride; N,N-dimethyl-N-octadecyl-N-(dimethylbenzyl)ammonium chloride; N,N-dimethyl-N,N-didecylammonium chloride; N,N-dimethyl-N,N-didodecylammonium chloride; N,N,N-trimethyl-N-tetradecylammonium chloride; N-benzyl-N,N-dimethyl-N-(C_{12}-C_{18}-alkyl) ammonium chloride; N-(dichlorobenzyl)-N, -N-dimethyl-N-dodecylammonium chloride; N-hexadecylpyridinium chloride; N-hexadecylpyridinium bromide; N-hexadecyl-N,N,N-trimethylammonium bromide; N-dodecylpyridinium chloride; N-dodecylpyridinium bisulphate; N-benzyl-N-dodecyl-N,N-bis(beta-hydroxy-ethyl)ammonium chloride; N-dodecyl-N-benzyl-N,N-dimethylammonium chloride; N-benzyl-N,N-dimethyl-N-(C_{12}-C_{14}-alkyl) ammonium chloride; N-dodecyl-N,N-dimethyl-N-ethylammonium ethylsulphate; N-dodecyl-N,N-dimethyl-N-(1-naphthylmethyl)ammonium chloride; N-hexadecyl- N,N-dimethyl-N-benzylammonium chloride; N-dodecyl-N,N-dimethyl-N-benzylammonium chloride and 1-(3-chloroally)-3,5,7-triaza-1-azonia-adamantane chloride; urea derivatives such as 1,3-bis(hydroxymethyl)-5,5-dimethylhydantoin; bis(hydroxymethyl)urea; 3-(3,4-dichlorophenyl)-1,1-dimethylurea; 3-(4-isopropylphenyl)-1,1-dimethylurea; tetrakis (hydroxymethyl)acetylene diurea; 1-(hydroxymethyl)-5 .5-dimethylhydantion and imidazolidinylurea; amino compounds such as 1,3-bis(2-ethyl-hexyl)-5-methyl-5-aminohexahydro-pyrimidine; hexamethylenetetramine; 1,3-bis(4-aminophenoxy)propane; and 2-[(hydroxymethyl)-amino]ethanol; imidazole derivatives such as 1[2-(2,4-dichloro-phenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole; 2-(methoxycarbonyl-amino)-benzimidazole; nitrile compounds such as 2-bromo-2-bromomethylglutaronitrile, 2-chloro-2-chloro-methylglutaro-nitrile; 2,4,5,6-tetra-chloroisophthaladinitrile; thiocyanate derivatives such as methylene(bis)thiocyanate; tin compounds or complexes such as tributyltinoxide, chloride, naphthoate, benzoate or 2-hydroxybenzoate; isothiazolin-3-ones such as 4,5-trimethylene-4-isothiazolin-3-one, 2-methyl-4,5-trimethylene-4-isothiazolin-3-one, 2-methylisothiazolin-3-one, 5-chloro-2-methyl-isothazolin-3-one, benzisothiazolin-3-one; 2-n-butylbenzisothiazolin-3-one; 2-n-hexylbenzisothiazolin-3-one; 2-n-octylbenzisothiazolin-3-one; 2-(2-ethylhexyl)benzisothiazolin-3-one; 2-(2-ethylbutyl)benziothazolin-3-one; 2-(2-phenylethyl)benzisothiazolin-3-one; 2-methylbenzisothiazolin-3-one, 2-octylisothiazolin-3-one, 4,5-dichloro-2-octylisothiazolin-3-one; thiazole derivatives such as 2-(thiocyanomethylthio)-benzthiazole and mercaptobenzthiazole; nitro-compounds such as tris(hydroxymethyl)nitromethane; 5-bromo-5-nitro-1,3-dioxane and 2-bromo-2-nitropropane-1, 3-diol; iodine compounds such as iodo propynyl butyl carbamate and tri-iodo allyl alcohol; aldehydes and derivatives such as glutaraldehyde (pentanedial), p-chlorophenyl-3-iodopropargyl, formaldehyde and glyoxal; amides such as chloracetamide;

N,N-bis(hydroxymethyl)chloracetamide; N-hydroxymethyl-chloracetamide and dithio-2,2-bis(benzmethyl amide); guanidine derivatives such as 1.6-hexamethylene-bis [5-(4-chlorophenyl)biguanide]; thiones such as 3,5-dimethyltetrahydro-1,3,5-2H-thiodiazine-2-thione; triazine derivatives such as hexahydrotriazine and 1,3,5-tri-(hydroxyethyl)-1,3,5-hexahydrotriazine, 6-chloro-2,4-diethyl-amino-s-triazine and 4-cyclopropylamino-2-methylthio-6-t-butylamino-s-triazine; oxazolidine and derivatives thereof such as bis-oxazolidine; furan and derivatives thereof such as 2,5-dihydro-2,5-dialkoxy-2,5-dialkylfuran; carboxylic acids and the salts and esters thereof such as sorbic acid and 4-hydroxybenzoic acid and their salts and esters; phenol and derivatives thereof such as 5-chloro-2-(2,4-dichloro-phenoxy)phenol; thio-bis(4-chlorophenol) and 2-phenylphenol; sulphone derivatives such as diiodomethyl-paratolyl sulphone; 2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine and hexachlorodimethyl sulphone; thioamides such as dimethyldithiocarbamate and its metal complexes. ethylenebisdithiocarbamate and its metal complexes, 2-mercaptopyridine-N-oxide and its metal complexes, azoles such as hexaconazole, propiconazole, azoconazole, cypropconazole; the compounds of Formula (1) in EP 382 375, especially azoxystrobin and chlorphthalonil.

According to a fourth aspect of the present invention there is provided a method for detecting The Polymer comprising the steps:

(a) subjecting a sample containing The Polymer to a detection means whereby the presence of the chromophoric marker in The Polymer generates a detection signal; and

(b) calculating the concentration of The Polymer from the detection signal generated in step (a).

Preferably the detection means comprises fluorescence spectrometry or Raman spectrometry.

In a first preferred embodiment of the present method the chromophoric marker comprises a fluorescent group and the detection means is a fluorescence spectrometer comprising:

(i) a means for irradiating the sample containing The Polymer whereby the chromophoric marker is stimulated from a lower energy level to an excited state;

(ii) a means for detecting fluorescent radiation emitted by the chromophoric marker when the marker spontaneously returns to a lower energy level; and

(iii) a means for generating a detection signal upon detection of the fluorescent radiation.

Preferably the means for irradiating the sample containing The Polymer comprises a source of electromagnetic radiation which emits at wavelengths within the absorbtion band of the chromophoric marker. It is especially preferred that the irradiation means is a

laser with a peak wavelength which is within the main absorbtion band of the chromophoric marker.

The preferred means generating a detection signal is a photodetector, for example a silicon photodiode or a charge coupled array. Upon detection of fluorescent radiation from the chromophoric marker the photodetector generates a voltage which is proportional to the intensity of fluorescent radiation generated by the sample. The intensity of fluorescent radiation is proportional to the concentration of the chromophoric marker present in the sample. Accordingly, the concentration of The Polymer can be calculated based upon the magnitude of the voltage signal generated by the photodetector.

In a second preferred embodiment of the present method the detection means comprises a Raman spectrometer.

When monochromatic light irradiates a sample most of the light is scattered elastically with no interaction. However, a small fraction of the incident light interacts with the sample causing fluorescence and inelastic scattering known as the Raman effect. The inelastically scattered radiation contains bands characteristic of the material being irradiated and is called the Raman spectrum.

The Raman effect is very weak and is often swamped by the fluorescence effect. However, the Raman spectrum can be greatly enhanced by tuning the wavelength of the incident radiation to a chromophore in a molecule. This is called Resonance Raman (RR). The strength of the spectrum can also be greatly increased by examining a molecule on a specific silver surface. This is known as Surface Enhanced Raman Spectroscopy (SERS).

The combining of Resonance Raman with SERS gives an increase in detection sensitivity. This combined effect is called Surface Enhanced Resonance Raman Spectroscopy (SERRS). Another advantage of SERRS is that fluorescence is greatly reduced or quenched and therefore masking of the Raman Spectrum caused by fluorescence is reduced. To perform SERRS the sample of interest is mixed with a silver colloid. irradiated with a monochromatic light and the SERRS spectrum measured. The spectrum is very characteristic of the chromophore of the material examined and the strength is dependent on the concentration of material present.

Thus, in the present method the concentration of The Polymer in the sample is determined by measuring the Raman spectrum generated by the chromophoric marker and the intensity thereof, preferably using the SERRS method as hereinbefore described. The concentration of The Polymer is then determined from the intensity of the spectrum.

An example of a suitable Raman spectrometer is disclosed in US 5,751,415 which is incorporated herein by reference thereto.

According to a fifth aspect of the present invention there is provided a method for maintaining the concentration of The Polymer according to the first aspect of the invention in a medium at or above a target concentration comprising the steps:

(a) measuring the concentration of The Polymer in the medium using the method according to the fourth aspect of the present invention;

(b) comparing the measured concentration with the target concentration; and

(c) adding a sufficient quantity of further antimicrobial polymer to the medium to maintain the concentration of The Polymer in the medium at or above the target concentration.

The medium in this aspect of the invention is preferably an aqueous medium, more preferably water from a swimming pool. The preferred methods for measuring the concentration of The Polymer in step (a) are the preferred methods hereinbefore described in relation to the fourth aspect of the present invention. and especially a method which uses fluorescence spectrometry to detect the chromophoric marker in The Polymer.

The target concentration in the present method is preferably the minimum concentration of The Polymer required to prevent antimicrobial growth in the medium. Accordingly, when The Polymer is a polymeric biguanide the target concentration will typically be from 10 to 30ppm.

Preferably steps (a) to (c) of the present process are automated such that the concentration of The Polymer present in the medium is automatically maintained at or above the target level. Automation is particularly useful for the protection of swimming pools because the concentration of antimicrobial materials in swimming pools can change quickly, for example through contamination of the swimming pool, or by dilution with fresh water.

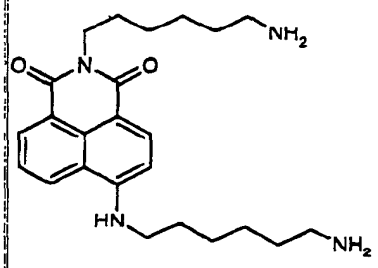
When the present method is automated it is preferred that step (b) generates an alarm signal when the concentration measured in step (a) falls to or below the target concentration. The alarm signal is then used to activate step (c) of the method and thereby increase the concentration of The Polymer. Preferably the additional antimicrobial polymer in step (c) is added to the medium from a reservoir containing The Polymer.

It is preferred that the concentration of The Polymer in step (a) is constantly monitored because this enables any reduction in concentration below the target level to be quickly detected and thereby reduces the possibility of contamination of the medium through proliferation of micro-organisms in the medium.

The invention is further illustrated by the following examples in which all parts are by weight unless otherwise indicated.

Intermediate Preparation

Preparation of Chromophoric Marker N-(6-aminohexyl)-4-(6-aminohexylamino)-1,8-naphthalimide ("Marker 1")



Marker 1

Marker 1 was prepared as follows:

Stage (a) Preparation of N-acetylhexylendiamine

Hexamethylenediamine (90.64g; 0.6 mol) and acetamide (11.81g; 0.2 mol) were stirred and refluxed for 10 hours under nitrogen. The solution was cooled overnight and then distilled under vacuum <100°C as fast as possible. The N-acetylhexylenediamine was then separated from the resultant mixture by flash chromatography [SiO_2/ EtOH:25%aq.NH_3 (4:1)]. The title product was obtained as a white crystalline product (18.43g; 58% theory).

Stage (b): Preparation of N-(N-acetyl-6-aminohexyl)-4-(N-acetyl-6-aminohexylamino)-1,8-naphthalimide

4-Bromo-1,8-naphthalic anhydride (2.77g; 0.01mol) and N-acetylhexylendiamine (1.61g; 1.5 10^{-3}mol) were melted together and stirred for 1 hour until the mixture became a brown solid. The compound obtained was purified by chromatography [silica gel/ hexane, CH_2Cl_2 then CH_2Cl_2:MeOH (10:1; 5:1; 3:1)].

Stage (c): Preparation of N-(6-aminohexyl)-4-(6-aminohexylamino)-1,8-naphthalimide

Hydrochloric acid (250mL; 4M) was added to the product of stage (b) and the mixture was refluxed for 9 hours. The solution was cooled and neutralised with sodium carbonate. Most of the salt was precipitated with ethanol (2L), the solution filtered and the solvent evaporated. The resulting mixture was salt and a brown oil. Water (100mL) was added and the product precipitated. The product was extracted into CH_2Cl_2 (addition of a small amount of MeOH helped dissolved the product in the CH_2Cl_2 layer), dried over MgSO4 and evaporated to give Marker 1 as an orange solid (1.90g; 46% theory).

mp: 113.6-114.9°C. Marker 1 had the following NMR spectrum:

NMR ^1H (300mHz, $CDCl_3$) δ_H: 1.1-1.6 (16H, m, $4xCH_2+4xNH_2$), 1.7-1.9 (4H, m, $4xCH_2$), 2.7 (4H, m, $2xCH_2$-NH_2), 3.4 (2H, q, Ar-N-CH_2), 4.2 (2H, t,)N-CH_2), 5.3 (1H, t, Ar-NH-R), 6.7 (1H, d, ArH), 7.6 (1H, t, ArH), 8.1 (1H, d, ArH), 8.4 (1H, d, ArH), 8.5 (1H, d, ArH) ppm
m/z (ES-): 409 $[M-H]^-$ (100%), 204 $[M-2H]^{2-}$ (15)
m/z (ES+): 411 $[M+H]^+$ (90%), 227 (100)

Example 1

Co-polymer containing Marker 1 as an In-Chain Group

Hexamethylenediamine dihydrochloride (1.48g; $8x10^{-3}$mol), 2% aqueous ammonium chloride solution (0.5ml), HMBDA (2g; $8x10^{-3}$mol) and Marker 1 ($3.48x10^{-3}$g) were added to a boiling tube. The mixture was then heated at 160°C for 2 hours. The resulting product was then dissolved in water (4ml) to stop the co-polymerisation, and the temperature reduced to 70°C. More water (4ml) and Celite filter aid (0.3g) were added. The mixture was filtered and the volume made up to 15mL to give the title product as a 20% aqueous solution.

Examples 2 to 4 and Comparative Example A

Further antimicrobial polymers containing Marker 1 were prepared using the method described in Example 1, except the quantity of Marker 1 used in the co-polymerisation is as shown in Table 1. The number average molecular weight of the resulting copolymer (Mn) was measured using gel permeation chromatography. The polymer of Comparative Example A did not contain Marker 1.

Table 1

Example	% weight of Marker 1 (vs. Total weight)	Mass of Marker 1 (g)	Mn
1	0.1	$3.48\ 10^{-3}$	931.2
2	0.5	$6.96\ 10^{-3}$	841.4
3	1	$3.48\ 10^{-2}$	913.7
4	5	$6.96\ 10^{-2}$	843.0
Comparative Example A	0	0	878.3

Example 5

Anti-Microbial Effect

The Minimum Inhibitory Concentration (MIC) against a range of fungi and bacteria were determined for each of the antimicrobial copolymers prepared in Examples 1 to 4 and Comparative Example 1. The MIC results are shown in Table 2:

Table 2

Example	Fungi	Bacteria						
	Ca	Pp	Bs	Ec	Pa	Sa	Ps.fl	K.pn
1	62.5	32	0.25	0.25	16	0.25	8	1
2	62.5	32	0.25	0.25	32	0.25	8	1
3	32	32	0.25	0.25	16	0.25	8	1
4	62.5	32	0.25	0.25	16	0.25	8	1
Comparative Example A	62.5	32	0.25	0.25	16	0.25	8	1
Marker 1	999	999	500	500	999	32	500	999

In Table 2 the following abbreviations are used:

Ca	Candida albicans	NCYC 10231
Bs	Bacillus subtilis	NCIB 1650
Ec	Escherichia coli	NCIB 9132
Psa	Pseudomonas aeruginosa	NCIB 10421
Sa	Staphylococcus aureus	NCIB 9518
Pp	Penicillium funiculosum	IMI 114933
Psfl	Pseudomonas fluorescens (with Lux AB gene)	our ref D481
Kpn	Klebsiella pneumoniae	ATCC 4352

Table 2 clearly shows that the presence of Marker 1 has no marked effect on the MIC compared to the polymer of Comparative Example A which does not contain Marker 1.

Table 2 also shows that Marker 1 itself has little or no antimicrobial effect compared to the copolymers containing it.

Further tests showed there was no effect on the speed of kill of Examples 1 to 4 containing Marker 1 compared to the poly(hexamethylenebiguanide) itself and which is free of Marker 1.

Fluorescent Detection

A 20% solution of the antimicrobial polymer prepared in Example 3 (1% of Marker 1) was diluted several times with distilled water to give a range of concentrations of from 1 to $10^{-6}gL^{-1}$ of the antimicrobial polymer.

The solution containing $1gL^{-1}$ of the antimicrobial polymer was analysed by UV/Vis spectroscopy (Perkin-Elmer, Lambda 15, UV/Vis spectrometer). An absorbance peak was found at 456nm. The same analysis on a solution containing 1g/L of the polymer of Comparative Example A (no Marker 1 present) showed no peak at this wavelength.

Using a fluorescence spectrometer (Perkin-Elmer, LS-5B, luminescence spectrometer), the lambda max. excitation wavelength was found to be 452nm and the emission spectrum was scanned between 475 and 800nm. The marker maximum emission was 532nm. All the solutions were scanned and it was found that the detection limit for this particular peak was for the $10^{-6}gL^{-1}$ solution of The Polymer according to the present invention.

CLAIMS

1. An antimicrobial polymer, characterised in that it carries a covalently bound chromophoric marker.

2. An antimicrobial polymer according to claim 1 wherein the antimicrobial polymer is a cationic antimicrobial polymer.

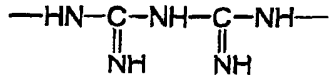
3. An antimicrobial polymer according to either claim 1 or claim 2 wherein chromophoric marker comprises a chromophoric group which has a major absorption and/or emission band in the range of from 390 to 1100 nm.

4. An antimicrobial polymer according to any one of the preceding claims wherein the chromophoric group is a fluorescent group.

5. An antimicrobial polymer according to any one of the preceding claims wherein the chromophoric marker is covalently bound to the antimicrobial polymer as a pendant group or a terminal group on the polymer chain, or as an in-chain group in the polymer chain.

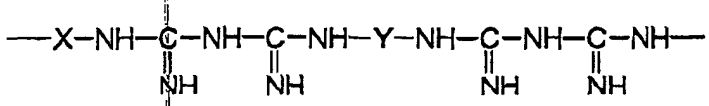
6. An antimicrobial polymer according to any one of the preceding claims wherein the chromophoric marker is present as a terminal or pendant group on the polymer chain and the antimicrobial polymer to which the chromophoric marker is bound is an antimicrobial poly(quaternary ammonium) compound, a polymeric guanide or a polymeric biguanide.

7. An antimicrobial polymer according to claim 6 wherein the antimicrobial polymer to which the chromophoric marker is bound is a polymeric biguanide which contains at least one biguanide unit of Formula (3):



Formula 3

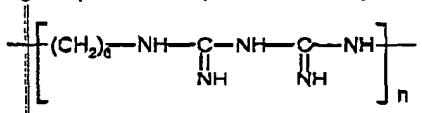
8. An antimicrobial polymer according to claim 7 wherein the polymeric biguanide is a linear polymeric biguanide which has a recurring polymeric unit represented by Formula (4)



Formula (4)

wherein X and Y may be the same or different and represent bridging groups in which, together, the total number of carbon atoms directly interposed between the pairs of nitrogen atoms linked by X and Y is not less than 9 and not greater than 17.

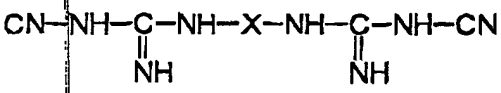
9. An antimicrobial polymer according to claim 8 wherein the polymeric biguanide is a mixture of poly(hexamethylenebiguanide) polymer chains in which the individual polymer chains, excluding the terminal groups, are represented by Formula (5) and salts thereof:



Formula (5)

wherein the value of n is from 4 to 40.

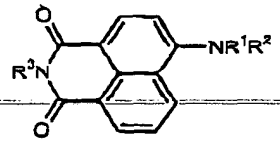
10. An antimicrobial polymer according to any one of claims 1 to 5 obtainable by co-polymerising a chromophoric marker, a bisdicyandiamide having the formula:



and a diamine H_2N-Y-NH_2, wherein X and Y are as defined in claim 8.

11. An antimicrobial polymer according to claim 10 obtainable by co-polymerising hexamethylenediamine, hexamethylene-1,6-bis dicyandiamide and a chromophoric marker.

12. An antimicrobial polymer according to claim 10 or claim 11 wherein the chromophoric marker is of the Formula (2):



Formula (2)

wherein:

R^1 is H or C_{1-6}-alkyl optionally substituted by amino, hydroxy or mercapto; and

R^2 and R^3 are, independently, alkyl substituted by a reactive functional group.

13. An antimicrobial polymer according to claim 10 obtainable by co-polymerising hexamethylenediamine, hexamethylene-1,6-bis dicyandiamide and 4-bromo-1,8-naphthalic anhydride.

14. A compound of the Formula (2) as defined in claim 12.

15. A composition comprising antimicrobial polymers at least one of which is an antimicrobial polymer according to any one of claims 1 to 13.

16. A composition comprising a carrier and an antimicrobial polymer according any one of claims 1 to 13 or a composition according to claim 15.

17. A method for inhibiting microbiological growth on, or in, a medium which comprises treating the medium with an antimicrobial polymer according to any one of claims 1 to 13 or a composition according to claim 15 or claim 16.

18. A method for detecting an antimicrobial polymer comprising the steps:
(a) subjecting a sample containing an antimicrobial polymer according to any one of claims 1 to 13 to a detection means whereby the presence of the chromophoric marker in the antimicrobial polymer generates a detection signal; and
(b) calculating the concentration of the antimicrobial polymer from the detection signal generated in step (a).

19. A method according to claim 18 wherein the detection means comprises fluorescence spectrometry, Raman spectrometry or surface enhanced resonance Raman spectrometry.

20. A method for maintaining the concentration of an antimicrobial polymer according to any one of claims 1 to 13 in a medium at or above a target concentration comprising the steps:
(a) measuring the concentration of the antimicrobial polymer in the medium using the method according to claim 18 or claim 19;
(b) comparing the measured concentration with the target concentration; and

(c) adding a sufficient quantity of further antimicrobial polymer to the medium to maintain the concentration of the antimicrobial polymer in the medium at or above the target concentration.